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Further Studies on O_2-Resistant Photosynthesis and Photorespiration in a Tobacco Mutant with Enhanced Catalase Activity

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ABSTRACT

The increase in net photosynthesis in M_4 progeny of an O_2-resistant tobacco (*Nicotiana tabacum*) mutant relative to wild-type plants at 21 and 42% O_2 has been confirmed and further investigated. Self-pollination of an M_3 mutant produced M_4 progeny segregating high catalase phenotypes (average 40% greater than wild type) at a frequency of about 60%. The high catalase phenotype cosegregated precisely with O_2-resistant photosynthesis. About 25% of the F_1 progeny of reciprocal crosses between the same M_3 mutant and wild type had high catalase activity, whether the mutant was used as the maternal or paternal parent, indicating nuclear inheritance. In high-catalase mutants the activity of NADH-hydroxypyruvate reductase, another peroxisomal enzyme, was the same as wild type. The mutants released 15% less photorespiratory CO_2 as a percent of net photosynthesis in CO_2-free 21% O_2 and 36% less in CO_2-free 42% O_2 compared with wild type. The mutant leaf tissue also released less $^{14}CO_2$ per [1-^{14}C]glycolate metabolized than wild type in normal air, consistent with less photorespiration in the mutant. The O_2-resistant photosynthesis appears to be caused by a decrease in photorespiration especially under conditions of high O_2 where the stoichiometry of CO_2 release per glycolate metabolized is expected to be enhanced. The higher catalase activity in the mutant may decrease the nonenzymatic peroxidation of keto-acids such as hydroxypyruvate and glyoxylate by photorespiratory H_2O_2.

In a recent paper (23), I described the selection of tobacco plants with O_2-resistant photosynthesis that differed in their characteristics from any previously reported. These plants showed a steady increase in net photosynthesis relative to wild-type plants on raising the O_2 level from 1% O_2 (where photosynthesis was the same) to 21% O_2 to 42% O_2 at 250 to 350 μL CO_2 L^{-1} and 30°C. Net CO_2 assimilation averaged about 15% greater than wild type in whole plants and leaf discs in 21% O_2. After two successive selfings of O_2-resistant plants, about 50% of the M_3 progeny showed O_2-resistant photosynthesis. Leaves of these plants averaged 40% greater catalase activity than wild type while glycolate oxidase activity, a closely related peroxisomal enzyme, was the same.

The results (23) were consistent with the view that the O_2-resistant plants released less photorespiratory CO_2 than wild type under conditions of high photorespiration relative to net photosynthesis, namely higher than normal O_2 and temperatures of 30°C or higher. There is evidence that high O_2 and high temperatures increase greatly the stoichiometry of CO_2 release relative to the flux of the photorespiratory pathway (3–5). Values above the 'expected' 25%, which would occur during the conversion of 2 glycine to produce serine and CO_2, would then be observed. The hypothesis developed was that the 'excessive' CO_2 losses above the 25% expected, during conditions of high photorespiration relative to net photosynthesis, resulted from the peroxidation of glyoxylate and especially hydroxypyruvate by photorespiratory H_2O_2 (3, 19). Enhanced catalase activity could therefore diminish excess loss of CO_2 by reducing the level of peroxisomal H_2O_2.

In this study, the M_4 progeny of an O_2-resistant plant have been examined, and it is shown that high catalase activity cosegregates precisely with O_2-resistant photosynthesis, while siblings with normal catalase activity have photosynthetic characteristics typical of wild type. Progeny of reciprocal crosses of an O_2-resistant M_3 plant with wild type have been studied and show that the high catalase phenotype results from a nuclear mutation. Evidence in support of the view that the O_2-resistant mutants have a decreased photorespiratory CO_2 release under conditions of high photorespiration is also presented.

MATERIALS AND METHODS

Plants and Photosynthesis Measurements

Tobacco (*Nicotiana tabacum*, L. cv Havana Seed) was grown in a greenhouse as previously described (23), and experiments were conducted on M_4 progeny of an M_3 plant with O_2-resistant photosynthesis originally derived from the M_1 plant designated as 42–12F (Fig. 1 in ref. 23). Studies were also conducted on progeny of reciprocal crosses made with the same M_3 plant.

Photosynthesis experiments were conducted with 18 1.6-cm leaf discs (0.36 dm^2 per leaf surface, about 720 mg fresh weight) floated upside down on a thin layer of water in a Petri plate. When comparing photosynthesis at 21 and 42% O_2 in segregating M_4 progeny, rates of net CO_2 uptake were measured at an irradiance of 500 μmol photons m^{-2} s^{-1} using an open system in 1 L Plexiglas chambers continuously flushed with a water-saturated gas of fixed composition at about 30°C. In experiments in which photorespiratory release was also measured and when $^{14}CO_2$ arising from [1-^{14}C]lycolate was collected, the leaf discs were placed in a 90 ml jacketed Plexiglas chamber at 650 μmol photons m^{-2} s^{-1} and leaf

temperatures were controlled within 0.1° of 30°C by adjusting the temperature-controlled liquid circulating around the chamber (12).

Enzyme Assays

To assay catalase activity, one leaf disc (2.01 cm^2) from near the leaf tip, frequently taken from the same sample used for photosynthetic measurements, was ground in a microcentrifuge tube fitted with a plastic pestle (Kontes, Vineland, NJ) containing 0.75 mL of ice-cold 0.05 M K phosphate buffer (0.05 M, pH 7.4) with 1.13 mg DTT (6). The suspension was centrifuged at 16,000*g* for 3 min, and the clear supernatant was assayed for catalase activity at 30°C by measuring the linear rate of decrease in absorbance of H_2O_2 at 240 nm. One unit is defined as the activity catalyzing the decomposition of 1 μmol H_2O_2 per min. Since high catalase activity was observed similarly on a fresh weight, protein, or leaf area basis (23), the specific activity was given as units per cm^2 leaf area. When comparing catalase activity in segregating plants, four discs from four different plants (three segregating progeny and one randomly selected wild type) were cut and extracted at the same time and assayed, and then another set was taken. In a single day, at least 15 segregating progeny and 5 wild-type plants were assayed. High catalase plants were defined as having activities at least 1 SD (67% of all observations) greater than the mean for wild-type plants assayed on that day.

Hydroxypyruvate reductase was assayed in the same extracts used for measuring catalase activity immediately after each set of catalase assays was completed. The reaction mixture (11, 18) consisted of 0.5 M K phosphate buffer (pH 6.4), 0.10 mL; 2 mM NADH, 0.10 mL; enzyme extract; and water to make the final volume 3.0 mL. After observing no oxidation of NADH at 30°C for 1 min, the reaction was initiated by the addition of 0.10 mL of 30 mM Li hydroxypyruvate, and the rate of NADH oxidation at 340 nm was determined. One unit is defined as the activity catalyzing the reduction of 1 μmol hydroxypyruvate per min.

Photorespiratory CO_2 and $^{14}CO_2$ Release from [1-^{14}C] Glycolate

To measure photorespiratory release in CO_2-free 21 and 42% O_2, leaf discs in the temperature-controlled 90 mL chamber were floated upside down on water (leaf temperature 30°C) and flushed with water-saturated 21% O_2/350 μL CO_2 L^{-1} at 1.75 L min^{-1} until steady rates of net photosynthesis were obtained. The flushing gas was then changed to water-saturated CO_2-free 21% O_2 and the constant rate of CO_2 release (from about 5–20 min) was measured. The procedure was then repeated with the same discs using 42% O_2 and the same CO_2 concentrations as before in the same sequence. At the end of the experiment, mean stomatal widths were determined from silicone rubber impressions made on two discs as previously described (23).

To investigate $^{14}CO_2$ release from [1-^{14}C]glycolate, steady rates of photosynthesis by 18 leaf discs floating upside down on water were first attained at 650 μmol photons m^{-2} s^{-1} leaf temperature 30°C, while the discs were flushed at 1.0 L min^{-1} with 21% O_2/350 μL CO_2 L^{-1}. Most of the water was then removed with a hypodermic syringe while the discs remained in the light and 180 μL of 10 mM K [1-^{14}C]glycolate (Amersham) (pH 5.0), containing 427,000 dpm (237,000 dpm/μmol) was quickly added next to the discs. Steady state photosynthesis was restored within several minutes, and in all experiments was identical in rate to that observed before addition of the tracer radioactive glycolate. The $^{14}CO_2$ released in the gas stream was collected in two traps in series each containing 70 mL of 2 M ethanolamine (3). Samples of the ethanolamine were removed at 15 or 20 min intervals from the first trap to determine that the rates of $^{14}CO_2$ release were constant, and from traps one and two after 60 min, from which a correction was made based on the efficiency of trapping (3). The experiments were terminated after 60 min, the discs were killed in 20 mL boiling 20% ethanol containing 10 mM $NaHSO_3$, and the killed discs homogenized and made to a known volume. Small samples were removed to determine total ^{14}C by scintillation counting. The homogenate was centrifuged at high speed and the residue washed twice by centrifugation. Radioactive glycolate was determined by elution of the extract on a column of Dowex-1 acetate ion exchange resin with 4 N acetic acid (17). The ^{14}C-glycolate metabolized was calculated from the total ^{14}C in the homogenate minus the ^{14}C remaining in glycolic acid.

RESULTS

Cosegregation of O_2-Resistant Photosynthesis with High Catalase Activity

Previous work demonstrated that M_3 progeny of an O_2-resistant tobacco line averaged about 40% greater catalase activity on a fresh weight, protein, or leaf area basis (23). This correlation was further tested in M_4 progeny (Table I). Net photosynthesis was determined in atmospheres containing 250 μL CO_2 L^{-1} at 21% O_2 and 42% O_2 in six segregants previously identified as having high catalase activity and in six segregants with normal (wild-type) catalase activity. In every case the segregants with high catalase activity, which was determined from the same sample of leaf discs used for the photosynthesis measurements, showed a sizeable increase in the net photosynthesis ratio of segregant/wild-type on going from 21 to 42% O_2. Plants with high catalase activity averaged 40% more than wild type. Segregants with normal catalase activity, on the other hand, did not differ from wild type.

Inheritance of High Catalase Activity in M_4 Progeny and in Crosses with Wild Type

Catalase activities were assayed in leaf discs taken from M_4 seedling progeny derived from self-pollination of an M_3 mutant (Table II). High catalase activity was found in 25 out of 42 plants assayed (60%). Since O_2-resistant photosynthesis is perfectly correlated with high catalase activity (Table I), one can assume that about 60% of the progeny in this generation had O_2-resistant photosynthesis as well. This probably represents an increase from the M_2 and M_3 generation, in which fewer progeny showed O_2 resistance (23).

Reciprocal crosses between the same M_3 mutant and a wild-

Table I. *Cosegregation of O_2-Resistant Photosynthesis with High Catalase Activity in M_4 Progeny of an O_2-Resistant Plant*

Net photosynthesis and catalase activity were determined on the same day on leaf discs cut from the same leaf (6–13 g fresh weight) of segregating plants previously identified as having high or wild-type enzyme activity. Each of the segregating progeny was compared with a randomly selected wild-type plant. A two-way analysis of variance was conducted for net photosynthesis at 21 and 42% O_2 in leaf discs of segregating *versus* wild-type plants. In each comparison, at least five determinations were made under steady-state conditions.

Individual Plant Assayed	Net Photosynthesis		Catalase Activity
	21% O_2	42% O_2	
	Segregant/WT		
Segregants with high catalase activity			
1	1.07 **	1.23 **	1.41
2	0.99 NS	1.10 NS	1.38
3	1.16 ***	1.47 ***	1.58
4	0.95 **	1.27 **	1.31
5	1.09 *	1.25 **	1.32
6	1.05 NS	1.18 **	1.39
Mean six plants	1.05	1.25	1.40
Segregants with normal catalase activity			
1	0.99 NS	0.92 NS	1.05
2	0.84 **	0.86 **	0.83
3	0.98 NS	1.02 NS	0.97
4	0.76 **	0.78 *	0.95
5	0.88 *	0.86 *	0.96
6	0.95 NS	0.89 NS	1.09
Mean six plants	0.90	0.89	0.98

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

type parent produced F_1 progeny segregating high catalase phenotypes at a frequency of about 25% regardless of the direction of the cross (Table II).

Catalase and Hydroxypyruvate Reductase Activities in High Catalase Mutants

I reported previously that plants with high catalase had normal glycolate oxidase activity (23). To further establish that high catalase levels do not reflect a general alteration in activity of peroxisomal enzymes, the activity of NADH-hydroxypyruvate reductase (15) was determined in the same extracts used to assay catalase. Both enzymatic rates were determined in M_4 progeny ($n = 7$) with high catalase, each on two separate days, and in randomly selected wild-type plants (Table III). Mutants averaged about 40% higher catalase activity than wild type, and an analysis of variance showed the difference was very highly significant ($P < 0.001$). However, hydroxypyruvate reductase activity was the same in wild-type and mutant plants.

Photorespiratory CO_2 Release in Wild Type and Mutants with High Catalase Activity

The hypothesis that O_2-resistant photosynthesis described here is caused by a decrease of photorespiratory CO_2 release was tested by measuring CO_2 efflux in light in a rapid stream of CO_2-free 21 and 42% O_2. The advantages and limitations

Table II. *Segregation for High Catalase Activity in Progeny of an O_2-Resistant M_3 Plant and in Reciprocal Crosses between the M_3 Plant and Wild-Type (WT)*

Catalase (CAT) assays were conducted in extracts of leaf discs of greenhouse-grown leaves (2–5 g fresh weight). The month in which the assays were conducted is shown for each planting. Plants with high catalase had activities 1 SD or greater above the means of wild-type plants assayed on the same day.

Planting	Number of Plants with High CAT	Number of Plants Tested	High Catalase Plants
			%
M_4 progeny			
1 (November)	10	16	
2 (February)	8	14	
3 (May)	7	12	
Total	25	42	60
(M_3 mutant × WT) progeny			
1 (December)	4	16	
2 (February)	5	18	
Total	9	34	26
(WT × M_3 mutant) progeny			
1 (December)	3	8	
2 (February)	3	16	
Total	6	24	25

of this assay of photorespiration have been discussed by me earlier (20). It has been used as a photorespiration assay in experiments with a photorespiratory mutant of *Arabidopsis* by others who concluded that the stoichiometry does not increase (14). The CO_2-efflux in CO_2-free 21% O_2 was shown to nearly double from 13 to 28°C as a percent of net photosynthesis in wheat leaves (10), consistent with the response of photorespiratory CO_2 in other assays.

Table IV shows results with four high-catalase M_4 mutants and four randomly selected wild-type plants regarding net photosynthesis in 21 and 42% O_2 and their photorespiratory CO_2 release in CO_2-free 21 and 42% O_2. The net photosynthesis ratio in the mutants/wild type increased greatly on going from 21 to 42% O_2 demonstrating their O_2 resistance. In 42% O_2, the mean absolute efflux of CO_2 was greater in wild type in three of the four experiments, and in experiment 4 it was significantly greater ($P < 0.001$). As a percent of net photosynthesis, there was a regular threefold increase in the photorespiratory CO_2 released per unit of net photosynthesis in wild-type, and a twofold increase in mutant leaf discs. In 21% O_2, the photorespiratory CO_2 released by the mutant averaged 15% less on a net photosynthesis basis than wild type ($P < 0.05$), while in 42% O_2, the photorespiratory CO_2 released by the mutant averaged 36% less than wild type ($P < 0.001$). Thus, O_2 resistance could be associated with decreased photorespiration as a percent of net photosynthesis, especially at higher O_2 levels.

Since such results on photorespiratory CO_2 release could have resulted from increased stomatal diffusive resistance in the mutant, at the end of each experiment stomatal widths were determined on two leaf discs. Stomatal numbers per unit leaf area were the same in wild-type and M_3 mutant plants (23). It was found (Table IV) that stomatal widths were the

Table III. *Comparison of Catalase (CAT) and Hydroxypyruvate Reductase (HPR) Activities in Seven Progeny from the Third Selfing of an O_2-Resistant Plant and in Wild-Type Plants (WT)*

Both enzymes were assayed in the same extract of a leaf disc cut from plants earlier identified as having high CAT activity. The extracts were made from each plant twice, each time on a different day, and compared with assays of extracts from randomly selected wild-type plants prepared at the same time. An analysis of variance was conducted on assays from high-CAT plants *versus* wild-type.

Mean CAT Activity		CAT Activity	Mean HPR Activity		HPR Activity
High CAT plants	WT plants		High CAT plants	WT plants	
units/cm²		*high CAT/WT*	*units/cm²*		*high CAT/WT*
288 ***	210	1.37	0.703 NS	0.721	0.98

*** $P < 0.001$.

same or greater in the mutants than wild type, thus ruling out this explanation of the results.

Release of $^{14}CO_2$ from Tracer [1-^{14}C]Glycolate

If O_2 resistance is associated with decreased photorespiration, one should obtain less $^{14}CO_2$ release from [1-^{14}C]glycolate supplied to intact leaf tissue which would label C-1 of glycine as well as glyxoylate and hydroxypyruvate. To test this hypothesis under physiological conditions, the label was provided to leaf discs being flushed with 21% O_2/350 μL CO_2 L^{-1} at 30°C and the $^{14}CO_2$ released was measured with time.

In all experiments (Table V) the rate of $^{14}CO_2$ produced was constant with time, indicating that the photorespiratory pools equilibrated rapidly with the radioactive glycolate. The high catalase M_4 mutants released about 19% less $^{14}CO_2$ than did the wild type, indicating that even at 30°C in 21% O_2 they produce less photorespiratory CO_2 per glycolate metabolized.

DISCUSSION

Although the pathway of photorespiration is now well defined, there appears still to be a great deal to learn concerning its regulation. One approach would be to increase the CO_2/O_2 specificity of ribulose bisphosphate carboxylase/oxygenase (8), but it is still uncertain whether this can be achieved. Earlier obervations that peroxisomal H_2O_2 could decarboxylate keto-acids such as glyoxylate and hydroxypyruvate (17, 19, 24) suggested, however, that a fixed stoichiometry of CO_2 release per glycolate oxidized was unlikely. Peroxisomes contain large amounts of catalase, but it may be inefficient in removing low concentrations of hydrogen peroxide (2).

Hanson and Peterson (3–5) have made simultaneous measurements of photorespiration based on a stereochemical probe and the postillumination CO_2 exchange transient in tobacco leaf discs under a number of conditions well removed from the CO_2 compensation point. To reconcile the differing trends in their estimates (Fig. 6 in ref. 3), they assumed a variable stoichiometry. They concluded that at 25°C, 21% O_2, and 340 μL CO_2 L^{-1}, the stoichiometry was close to the expected 25% (5), however, raising the temperature to 32°C and the O_2 to

Table IV. *Net Photosynthesis (PS) and Photorespiratory CO_2 Release by Leaf Discs in CO_2-Free 21% O_2 and 42% O_2 in Wild-Type (WT) Plants and Mutants with High Catalase (CAT) Activity*

At the end of the experiments silicone rubber impressions were made on the exposed leaf disc surface to determine stomatal widths. Discs from a different WT and high CAT plant were compared on the same day in each experiment. Plants from a segregating population from the third selfing with high CAT activity relative to WT were identified 2 to 3 weeks prior to these experiments in greenhouse-grown plants. An analysis of variance compared mean net photosynthesis and mean stomatal widths in WT with high CAT plants.

Experiment No.[a]	Plant Type	Net PS		CO_2 Release	
		21% O_2	42% O_2	CO_2-free 21% O_2	CO_2-free 42% O_2
		mg CO_2 dm^{-2} h^{-1}		*% net PS*	
1	WT	23.2	11.1	15.0	45.0
	CAT	29.3 ***	18.9 ***	12.9	26.5
	Ratio CAT/WT[b]	1.26	1.70		
2	WT	29.5	16.4	16.4	45.2
	CAT	31.9 NS	21.8 ***	13.5	30.6
	Ratio CAT/WT	1.08	1.33		
3	WT	27.6	17.6	18.8	40.8
	CAT	36.6 ***	27.3 ***	14.2	27.3
	Ratio CAT/WT	1.33	1.55		
4	WT	34.5	17.0	16.8	48.5
	CAT	32.1 NS	23.3 *	16.0	30.0
	Ratio CAT/WT	0.93	1.37		
	Mean, WT			16.8	44.9
	Mean, high CAT			14.2 *	28.6 ***

[a] In experiment 1 mean stomatal widths for WT and high CAT, respectively, were 1.0 μm and 1.2 μm *; in experiment 2, 3.7 μm and 2.8 μm NS; in experiment 3, 4.7 μm and 5.8 μm *; in experiment 4, 3.2 μm and 4.4 μm *. [b] The catalase activity ratio of plants with high CAT/randomly selected WT was 1.37 for the plant in experiment 1, 1.29 in experiment 2, 1.47 in experiment 3, and 1.37 in experiment 4. * $P < 0.05$; *** $P < 0.001$.

42% produced results consistent with a carbon ratio stoichiometry, CO_2/glycolate, of 55%. Thus, the stoichiometry of photorespiration may not be fixed. They pointed out (3) that the nonenzymatic peroxidation of hydroxypyruvate (which yields glycolate and CO_2) could result in a stoichiometry of 100% while that resulting from peroxidation of glyoxylate (yielding formate and CO_2) would be 25% unless the formate is also oxidized to CO_2. Hanson and Peterson (3) also suggested that increasing the catalase content of peroxisomes could decrease the stoichiometry of photorespiration under conditions where it exceeds 25%.

The mutant plants obtained in the M_4 generation averaged about 40% greater catalase activity than wild type and this activity cosegregated with O_2-resistant photosynthesis (Tables I and IV). Data in Table II show that about 60% of the M_4 progeny of an O_2-resistant M_3 plant had high catalase. However, only about 25% of the progeny of reciprocal crosses with wild type have high catalase, whether the mutant was used as the maternal or paternal parent. This indicates that high catalase is controlled by a nuclear gene.

Two other peroxisomal enzymes, glycolate oxidase (23) and NADH-hydroxypyruvate reductase (Table III), have the same activities in the mutant plants as in wild-type, indicating that

Table V. *Release of $^{14}CO_2$ from Tracer [1-^{14}C]Glycolate Supplied to Photosynthesizing Leaf Discs of Wild-Type and High-Catalase M_4 Tobacco in 21% O_2*

Steady rates of photosynthesis were first attained with leaf discs floated upside down on water, and [1-^{14}C]glycolate was quickly added to the discs. The experiments were terminated after 60 min and the ^{14}C-glycolate converted to other products was determined. The mean photosynthesis rates ± SD are given during the treatment with [1-^{14}C]glycolate and the number of determinations are shown in parentheses.

Plant Type	Mean Photosynthesis Rate	$^{14}CO_2$ Released	[1-^{14}C] Glycolate Metabolized	$^{14}CO_2$ Released/ [1-^{14}C] Glycolate Metabolized
	mg CO_2 dm^{-2} h^{-1}	*nmol h^{-1}*		
Wild-type				
1	30.4 ± 0.6 (5)	65.8	300	0.22
2	31.7 ± 1.2 (6)	61.2	304	0.20
High catalase				
1	32.4 ± 0.6 (4)	59.4	342	0.17
2	33.7 ± 1.7 (7)	56.1	333	0.17

the high catalase levels do not result from a general increase in activity of peroxisomal enzymes. This further supports the specific nature of the mutation.

Tobacco has at least two catalase isozymes (6, 7), including one primarily with catalatic activity (CAT-1) and one with enhanced peroxidatic activity (CAT-3). The tobacco mutants with enhanced catalase activity contain both isozymes, and on the basis of preliminary analyses it was not possible to determine whether their proportion was altered in comparison with wild type (E Havir, personal communication).

Besides the studies cited above, there are considerable data in the literature indicating that the stoichiometry of photorespiration is not fixed (1, 16, 17). The argument for a fixed stoichiometry is based on experiments which may have alternative interpretations. For example, Somerville and Ogren (14) described studies with a mutant of *Arabidopsis* that lacked the enzyme in the photorespiratory pathway converting glycine to serine and CO_2. This mutant can therefore make glyoxylate but presumably cannot make hydroxypyruvate. Leaves of the mutant released 30% as much photorespiratory CO_2 into CO_2-free 50% O_2 as wild type from peroxidation of glyoxylate, and adding NH_4^+ suppressed CO_2 release from the mutant but had no effect on the wild type. They concluded that glyoxylate could be oxidized to CO_2 only when there were insufficient amino groups for transamination of glyoxylate to glycine. The experiments actually demonstrated that NH_4^+ does not limit the conversion of glyoxylate to glycine in wild type and say nothing about how much peroxidation of glyoxylate takes place in wild type.

This appears to be another example (22) of the limitations of deletion mutants, which accumulate metabolites in unusual concentrations and thereby alter metabolism, for extrapolating to rates of reactions taking place in wild type. These experiments were conducted with a mutant that does not produce hydroxypyruvate which is the keto-acid substrate for peroxidation most likely to produce a higher stoichiometry.

Kendall *et al.* (9) have also concluded that their experiments with a catalase-deficient barley mutant were consistent with a fixed stoichiometry of photorespiration. They proposed that in the near absence of catalase, one should expect a far greater proportion of $^{14}CO_2$ to be released as a percent of [1-^{14}C] glycolate metabolized. Their results showed less $^{14}CO_2$ was released in CO_2-free air by the mutant, consistent with their conclusion, but experiments in normal air showed a 17% greater release of $^{14}CO_2$ by the mutant (Table 2 in ref. 9). The latter results are similar to those in Table V which show wild-type plants, with 40% less catalase than the mutants, released 24% more $^{14}CO_2$ per ^{14}C-glycolate metabolized.

The initial selection of plants with O_2-resistant growth was made for the purpose of obtaining plants with a decreased rate of photorespiration (21, 23). The tobacco mutants described here averaged 15% less photorespiratory CO_2 release than wild type when expressed as a percent of net photosyntheis in 21% O_2 and 36% less in 42% O_2 (Table IV). The results were not caused by decreased stomatal diffusion of the mutants. The relative decrease in photorespiratory CO_2 per unit of net photosynthesis at higher O_2 is consistent with the hypothesis that the O_2-resistant photosynthesis in the mutants is caused by a decreased photorespiration, especially under conditions where the stoichiometry of photorespiration is expected to increase. The experiments in Table V, in which the photorespiratory pathway was labeled by supplying tracer quantities of [1-^{14}C]glycolate, show that even at 30°C in normal air, mutants with higher catalase activities released 19% less $^{14}CO_2$ than wild type. These results are also consistent with the mutant having a lower photorespiratory CO_2 release.

On successive selfings of an M_1 O_2-resistant plant the percent of progeny with O_2-resistance appears to have increased (23) to about 60% found in M_4 progeny (Table II). In the amphiploid *N. tabacum* pairing and recombination can take place between homeologous chromosomes of different genomes (13). Mutations may segregate into an array of heterozygous genotypes upon selfing if the chromosome can pair with its nonmutated partner in the other genome. Continued selection of resistant individuals for selfing would then be expected to gradually increase the frequency of the mutant gene(s). Eventually, one would expect to produce a population that breeds true for the resistance phenotype and may in the homozygous state display an even greater enhancement of catalase levels than has been observed in progeny of the M_3 and M_4 generations. Such mutants might show more O_2 resistance with still higher net CO_2 uptake per unit leaf area.

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